Aus der Klinik für Kleintiermedizin

der Veterinärmedizinischen Fakultät der Universität Zürich

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Arbeit unter der Leitung von: Prof. Dr. C. Reusch

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# Evaluation des NOVA CRT 8 Elektrolytanalyzers zur Messung von ionisiertem Kalzium und ionisiertem Magnesium bei Hund und Katze

## INAUGURAL-DISSERTATION

zur Erlangung der Doktorwürde

der Veterinärmedizinischen Fakultät

der Universität Zürich

vorgelegt von

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Prof. Dr. C. Reusch, Referentin

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**Für meinen Vater**

## Inhaltsverzeichnis

## Evaluation des NOVA CRT 8 Elektrolytanalyzers zur Messung von ionisiertem Kalzium und Magnesium bei Hund und Katze

## Zusammenfassung

Im Blut liegen die physiologisch sehr wichtigen Elektrolyte Kalzium und Magnesium proteingebunden, als freie Ionen und als Komplexe mit verschiedenen Substanzen vor. Nur die ionisierte Fraktion ist biologisch aktiv und wird über verschiedene Rückkoppelungsmechanismen in einem sehr engen Konzentrationsbereich gehalten.
Mit dem Nova CRT 8 Analyzer, welcher in der Humanmedizin schon seit längerer Zeit verwendet wird, ist es möglich Kalzium und Magnesium in der ionisierten, freien Form zu messen. Damit können klinisch relevante Elektrolytabweichungen besser erfasst werden.
Die Studie besteht aus 3 Teilen:
Im ersten Teil wurde der Analyzer anhand von Untersuchungen zur Präzision, Linearität und analytischen Genauigkeit evaluiert. Die Ergebnisse dieser Untersuchungen zeigen, dass der NOVA CRT 8 sehr präzise und genau arbeitet.
Im zweiten Teil wurden Untersuchungen zur Probenhandhabung und –stabilität durchgeführt. Es konnte gezeigt werden, dass Serumproben streng anaerob behandelt werden müssen, damit verlässliche Ergebnisse erzielt werden. Vergleichsuntersuchungen von Vollblut, Serum und Plasma ergaben, dass die einzelnen Blutprobentypen zu unterschiedlichen Messergebnissen führen. Die Ursache der Abweichungen der Messergebnisse in Vollblut und Plasma liegt an den verwendeten Antikoagulantien. Die Stabilitätsuntersuchungen zeigten, dass sowohl ionisiertes Kalzium wie auch ionisiertes Magnesium in anaerob behandeltem Serum, welches bei Zimmertemperatur gelagert wurde, über die Dauer eines Arbeitstages stabil bleiben. Durch Aufbewahrung der Proben bei 4° C kann die Stabilität bezüglich des ionisiertem Kalziums verlängert werden, im Gegensatz dazu scheint das ionisierte Magnesium bereits nach 24 instabil zu werden.
Im dritten Teil wurden Referenzwerte für ionisiertes Kalzium und Magnesium beim Hund und bei der Katze erstellt. Dazu wurden 30 Hunde- und 36 Katzenserumproben von gesunden Tieren herangezogen.
Aus den Untersuchungen geht hervor, dass sich der NOVA CRT 8 Analyzer für die Bestimmung von ionisiertem Kalzium und ionisiertem Magnesium bei Hund und Katze gut eignet. Um verlässliche Resultate erzielen zu können, müssen streng anaerob behandelte

Serumproben verwendet werden. Bei Raumtemperatur gelagerte Proben sollten innerhalb von 8 Stunden untersucht werden.

Die Arbeit liegt als englischsprachiges Manuskript vor, da sie in Form von 2 Publikationen in veterinärmedizinischen Fachzeitschriften eingereicht wurde.

# Evaluation of an electrolyte analyzer for measurement of ionized calcium and magnesium concentrations in dogs and cats

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# PART 1:

## Evaluation of an electrolyte analyzer for measurement of ionized calcium and magnesium concentrations in dogs

ABSTRACT

The goal of this study was to evaluate the NOVA CRT 8 electrolyte analyser for determination of concentrations of ionised calcium ($Ca_i$) and magnesium ($Mg_i$) in dogs, to determine the effects of sample handling, to determine the stability of samples and to establish analyser-specific reference ranges for $Ca_i$ and $Mg_i$ in healthy dogs. The precision, linearity and analytical accuracy of the NOVA CRT 8 analyser were good. The concentrations of $Ca_i$ and $Mg_i$ were significantly lower in aerobically handled serum samples than in those handled anaerobically. The concentrations of $Ca_i$ and $Mg_i$ differed significantly among whole blood, plasma and serum. In anaerobically handled serum, the concentration of $Ca_i$ was stable for 24 hours at 22ºC, for 48 hours at 4ºC and for 11 week at -20ºC. In contrast, the concentration of $Mg_i$ was stable for 8 hours at 22ºC but for less than 24 hours at 4ºC and for less than 1 week at -20ºC. In anaerobically handled serum from 30 dogs, the reference ranges were 1.20 – 1.35 mmol/L for $Ca_i$ and 0.42 – 0.58 mmol/L for $Mg_i$. The NOVA CRT 8 electrolyte analyser is suitable for determination of $Ca_i$ and $Mg_i$ concentrations in cats. Anaerobically handled serum samples stored at room temperature yield accurate results when analysed within 8 hours.

## INTRODUCTION

Calcium and magnesium are two essential electrolytes, which are important in various intracellular and extracellular functions as well as bone development.[4,15,18] In serum these cations may be ionized, protein-bound or complexed with substances such as phosphate, citrate or lactate. However, only the ionized fraction is biologically active and is maintained in a narrow concentration range, which is controlled by a number of feedback mechanisms.[5,18] For financial and technical reasons, most laboratories measure only the total concentration of calcium and magnesium. Although in many cases this offers important information about electrolyte abnormalities, it correlates poorly with the ionized form in conditions such as hypoproteinemia, hyperproteinemia, acid-base imbalance and renal insufficiency.[9,14,19,21] Thus, it would be advantageous to be able to directly measure the concentrations of ionized calcium ($Ca_i$) and magnesium ($Mg_i$). Only a few analyzers with ion-selective electrodes for the determination of $Ca_i$ and $Mg_i$ are available, including the NOVA CRT 8 [a], AVL 988/4 [b] and Microlyte 6 [c].[2] A number of studies reported that these recently developed analyzers produce reliable results[1,2,13,16,23], while other studies on the comparison of different analyzers showed that marked analyzer-specific variations exist[3,6,7,8]. For this reason it is necessary to use analyzer-specific reference ranges. Furthermore, in order to assure accurate results, specific techniques for sample collection and handling must be followed.[10,11,20,21]

The NOVA CRT 8 can measure the concentrations of $Ca_i$ and $Mg_i$ in whole blood, plasma and serum. A number of studies report that this analyzer is well suited for use in human medicine.[d,1,23] To the authors' knowledge it has not been evaluated in veterinary medicine. Therefore, the goals of this study were to evaluate the NOVA CRT 8 analyzer for its usefulness to determine $Ca_i$ and $Mg_i$ concentrations in dogs, to determine analyzer-specific reference ranges for $Ca_i$ and $Mg_i$ in healthy dogs and to investigate the effects of various external factors on the measurements obtained.

MATERIALS AND METHODS

**Analyzer** - The Nova CRT 8 Electrolyte Analyzer equipped with ion selective electrodes (ISE) is an instrument designed for simultaneous measurements of hematocrit, $Na^+$, $K^+$, pH, $Mg_i^{++}$ and $Ca_i^{++}$ in whole blood, plasma and serum. The analyzer provides calculated results for $Ca_i$ (measurement range 0.1 - 5.0 mmol/l) and $Mg_i$ (measurement range 0.1 – 2.5 mmol/l), normalized to a pH of 7.4. The equation used for this calculation is:

log [electrolyte] 7.4 = log [electrolyte] X – 0.24 (7.4 – X),

where X is the measured pH of the sample. In addition, the electrical signal from the $Mg^{++}$-selective electrode is adjusted by the signal from the $Ca^{++}$ electrode by an algorithm making use of the selectivity constant $K_{MgCa}$. The analyzer performs a 2-point calibration with two different $Ca^{++}$ and $Mg^{++}$ aqueous solutions. A sample volume of 180 µL is required and the measurement cycle is 55 s.[e]

**Dogs** - Thirty dogs owned by employees of the Faculty of Veterinary Medicine, University of Zurich, were used for this study. These dogs were considered healthy based on history, physical examination, complete blood count and biochemical profile. The dogs belonged to 17 different breeds and ranged in age from 1 to 13 years (mean 5.6 years). There were 7 intact female, 8 spayed female, 9 intact male and 6 castrated male dogs. Of these 30 dogs subgroups were used to study the influence of sample handling and storage conditions: six dogs were used to investigate effect of air, 7 dogs were used for comparison of different types of blood specimens and stability at 22° C, and different 7 dogs were used to study sample stability at 4° C and – 20°C. All 30 dogs served as controls to establish reference ranges for $Ca_i$ and $Mg_i$ concentrations.

**Precision, linearity and analytical accuracy** - For the determination of precision in a measurement series, two standard samples from the manufacturer[f], with a low ($Ca_i$ 0.59 – 0.83 mmol/l; $Mg_i$ 0.26 –0.42 mmol/l) and a high ( $Ca_i$ 1.50 – 1.90 mmol/l; $Mg_i$ 1.32 – 1.68 mmol/l) concentration of $Ca_i$ and $Mg_i$, and a canine serum sample with a mid-range ($Ca_i$ 1.12 mmol/l; $Mg_i$ 0.46 mmol/l) concentration of $Ca_i$ and $Mg_i$ were measured ten times within 10 minutes. For day-to-day precision, the manufacturer's two standard samples with a low and a high concentration of $Ca_i$ and $Mg_i$ were measured every 24 hours for 10 consecutive days. Samples were stored anaerobically at 4ºC.

For the determination of linearity, highly concentrated aqueous solutions of calcium (4.84 mmol/L) and magnesium (2.49 mmol/L) were prepared from crystalline calcium chloride[g] ($Ca\ Cl_2 \times 2\ H_2O$) and crystalline magnesium chloride[h] ($Mg\ Cl_2 \times 6\ H_2O$), respectively. These stock solutions were diluted with isotonic saline solution to make 50%, 25%, 12.5% and 6.25% solutions. For each solution, the concentration of $Ca_i$ and $Mg_i$ was measured with the analyzer and compared to the calculated values.

For determination of analytical accuracy $Ca_i$ and $Mg_i$ in different weighed in $Ca_i$ and $Mg_i$ aqueous solutions ( $Ca_i$ = 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 mmol/l; $Mg_i$ =0, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5 mmol/l) were measured. Different $Ca_i$ and $Mg_i$ concentrations were prepared by adding different amounts of either 0.1 molar calcium[i] or 0.1 molar magnesium[j] ion standard solution to 10 ml of isotonic saline. Direct measurements were compared to actual weighed in electrolyte concentrations.

**Effect of air** - To determine the effect of air on measurements, blood was collected from the jugular vein of 6 dogs. For each dog, the blood was then divided among 3 serum tubes[k] such that 100%, 50% and 25% of the tube was filled. In the 100% filled tubes any inclusion of air bubbles was specifically and diligently avoided to ascertain completely anaerobic conditions. Fifteen minutes later the samples were centrifuged at 7500 x g for 2- 5

minutes, followed immediately by measurement of serum concentrations of $Ca_i$ and $Mg_i$. The differences of measurements by different fillings were calculated.

**Comparison of $Ca_i$ and $Mg_i$ in whole blood, plasma and serum** - For determination of $Ca_i$ and $Mg_i$ in whole-blood, samples were collected from 7 dogs into a heparin-coated syringe[l] and immediately measured from the tip of the syringe . Thereafter, the remaining blood was placed in an Eppendorf serum tube[m] for harvesting plasma, excluding any air bubbles from entering the samples. Within 5 minutes a second blood sample was collected from each dog into a tube without anticoagulant for harvesting serum. All tubes were allowed to sit for 15 minutes, after which they were centrifuged at 7 500 x g for 2-5 minutes, before $Ca_i$ and $Mg_i$ were measured in plasma and serum.

**Stability of samples stored anaerobically at different temperatures** - Serum was placed as 0.5 ml aliquots into completely filled air-tight tubes[n]. From 7 dogs, serum was stored at room temperature (22ºC). Serum from different 7 dogs was stored either at refrigerator temperature (4ºC) or at freezer temperature (-20ºC). For all 3 temperature groups, the first measurement was performed immediately after centrifugation of the blood. For samples stored at 22ºC, additional measurements were made 1, 2, 3, 4, 8, 24 and 48 hours later. For samples stored at 4ºC, additional measurements were made every 24 hours for 5 days. For samples stored at –20ºC, additional measurements were made 1, 3, 11 and 26 weeks later. The frozen samples were thawed for 30 minutes at 22ºC before processing. The measurements obtained after storage were compared to the values of the first measurement. Samples were considered stable when the ANOVA did not reveal any significant differences from the first measurement.

**Reference values** - A blood sample was collected from the jugular vein of all 30 dogs and placed into an Eppendorf serum tube[m]. The tubes were filled completely, excluding any visible air bubbles, and closed tightly. After 10 to 15 minutes, the tubes were centrifuged at 7 500 x g for 2 – 5 minutes and the concentrations of $Ca_i$ and $Mg_i$ were immediately determined.

**Statistics** - Data were compiled using Excel 98 (Microsoft Inc.). Statistical analysis was performed using StatView 5.0 (SAS Inc., Wangen Dübendorf, Switzerland). Data were subjected to the QC-test for normality to confirm that they were sampled from a Gaussian distribution. Precision was calculated from coefficients of variation. Linearity was assessed using regression analysis and correlation coefficients. ANOVA was used for the comparison of multiple means and the Bonferroni-Dunn post test was used for the comparison of 2 individual means. Sample stability was analyzed using ANOVA for repeated measures. The Bonferroni-Dunn post test was used for comparison of values after storage to those at the start of storage. Reference ranges were defined as the range from the 5th to the 95th percentiles. Unless otherwise stated, all values are reported as mean ± SD and mmol/l. Differences were considered statistically significant when $P \leq 0.05$.

RESULTS

**Precision, linearity and analytical accuracy** - The variation coefficients for precision in a measurement series for the different concentrations of $Ca_i$ and $Mg_i$ ranged from 0.5 to 1.5%. Those for day-to-day precision ranged from 1.1 to 2.3% (Table 1).

For both $Ca_i$ and $Mg_i$, there was very good agreement between the calculated and measured concentrations in the different dilutions for the entire measurement range of the analyzer. The correlations and equations for the linear regressions for $Ca_i$ were $y = - 0.02 + 0.21\ x$, $R = 0.99$ and for $Mg_i$ were $y = 0.03 + 0.39\ x$, $R = 0.99$.

Direct measurements of $Ca_i$ and $Mg_i$ compared with the actual weighed in elecrolyte concentrations were linear in the measured range for both electrolytes. The correlations and equations for the linear regressions for $Ca_i$ were $y = 0.05 + 0.94\ x$, $R = 1.00$ and for $Mg_i$ were $y = 0.03 + 0.88\ x$, $R = 0.99$ (Fig. 1).

**Effect of air** - The concentrations of $Ca_i$ in tubes filled to 50% capacity (1.29 ± 0.05 mmol/l) and 25% capacity (1.26 ± 0.04 mmol/l) were significantly lower than the concentration in tubes that were completely filled (1.33 ± 0.06 mmol/l), $P < 0.01$. This was also true for the concentrations of $Mg_i$, which were 0.51 ± 0.06 mmol/l in completely filled tubes and 0.50 ± 0.06 and 0.48 ± 0.06 mmol/l in tubes filled to 50% and 25% capacity, respectively, $P < 0.01$ (Fig. 2).

**Comparison of $Ca_i$ and $Mg_i$ in whole blood, plasma and serum** - The concentration of $Ca_i$ was significantly lower in plasma (1.21 ± 0.18 mmol/l) compared to whole blood (1.26 ± 0.18 mmol/l) and serum (1.25 ± 0.18 mmol/l). The concentration of $Mg_i$ was significantly lower in serum (0.48 ± 0.07 mmol/l) compared to whole blood (0.54 ± 0.10 mmol/l) and plasma (0.53 ± 0.07 mmol/l; Fig. 3).

**Stability of samples stored anaerobically at different temperatures** - The concentration of $Ca_i$ was stable for 24 hours at 22ºC, for 48 hours at 4ºC and for 11 weeks at -20ºC. The concentration of $Mg_i$ was stable for 8 hours at 22ºC. After 24 hours at 4ºC and 1 week at -20ºC, the concentration of $Mg_i$ was significantly higher compared to the concentration before storage (Table 2).

**Reference values -** The reference range using anaerobically handled serum was 1.20 – 1.35 mmol/l for $Ca_i$ and 0.42 – 0.58 mmol/l for $Mg_i$ (Fig. 4) .

DISCUSSION

This study demonstrated that the NOVA CRT 8 analyzer is very suitable for the measurement of ionized calcium and magnesium in serum of dogs. Both the precision and linearity of the analyzer yielded good results. As well, our results supported those of other studies which concluded that specific handling, processing and storage of samples are crucial for accurate measurement of ionized electrolyte concentrations.[10,11,20,21]

Allowing serum to mix with air results in an increase in the serum pH, leading to a significant decrease in the concentration of $Ca_i$.[20] In our study, a significant decrease in the $Ca_i$ and $Mg_i$ concentrations occurred approximately 20 minutes after blood collection in tubes filled to 50% capacity compared to tubes that were filled completely. Hence, our findings also underline the importance of strictly anaerobic sample handling and storage.

The concentrations of $Ca_i$ and $Mg_i$ can be measured in whole blood, plasma or serum. However, anticoagulants used for the collection of whole blood and plasma may form complexes with electrolytes and thus, lower their concentrations.[17] It is also possible that ions contained in anticoagulants interfere with the electrodes resulting in erroneously high values.[11] In our study, the concentration of $Ca_i$ was significantly lower in plasma than in serum, which was possibly due to heparin binding with calcium ions.[17] Although whole blood samples also contained heparin, $Ca_i$ was higher in these samples compared to heparin plasma samples. Our only explanation for this difference is the time frame of exposure to heparin, as the whole blood samples were analyzed within seconds of collection, whereas heparin plasma samples were analyzed 20 minutes thereafter.

In contrast, $Mg_i$ was significantly lower in serum. The reason for the higher $Mg_i$ concentration in plasma and whole blood than in serum is also believed to be related to the anticoagulant. Lithium and zinc ions, both part of the anticoagulant, can interfere with the

magnesium electrodes and inflate the $Mg_i$ concentration. However, depending on the anticoagulant used false low or elevated results for $Mg_i$ may be obtained.[17]

Hence, serum is the specimen best suited for clinical use because there is no interference by an anticoagulant and the stability of ionized electrolytes appears to be better in serum than in whole blood.[19] However, in addition to strict anaerobic handling, the serum should be harvested as quickly as possible (less than 1 hour) to prevent glycolysis and lactate accumulation, which decreases the sample pH.[e]

The results of this study indicate that for in-house analysis, serum does not need to be refrigerated because the concentrations of $Ca_i$ and $Mg_i$ remain stable at room temperature for 8 hours. Refrigeration (4ºC) is advised for determination of $Ca_i$ concentration in serum in case samples can not be analyzed within 24 hours. If analysis will not be performed within 48 hours, the serum should be frozen (-20ºC). In contrast, the concentration of $Mg_i$ was significantly decreased after 24 hours when stored at 4ºC and after 1 week when stored at -20ºC. Based on the results of our study reliable results for $Mg_i$ can only be expected within 8 hours of collection, when kept at room temperature. For a more accurate determination of sample stability of $Mg_i$ stored at 4ºC or -20ºC, measurements in shorter time intervals would be necessary.

Reference ranges for $Ca_i$ and $Mg_i$ should be established with regard to species, age and sample type as well as to method of analysis.[19] Reference ranges for $Ca_i$ have not previously been established for the NOVA 8 electrolyte analyzer. Those obtained in this study were markedly lower (mean = 1.28 mmol/l) than those determined with another analyzer[o] (mean = 1.37 mmol/l)[14, 20]. Such analyzer-dependent differences have been described previously and are due to differences in analyzer components.[1,7] The reference range established for the serum $Mg_i$ concentration was in agreement with that of Mann et al. (1998), who used the same analyzer. This indicates that substantial differences in measurements do not occur among NOVA CRT 8 analyzers. In the future, technological features of different electrolyte

analyzers may possibly be harmonized thus eliminating the need for analyzer-specific reference ranges.[1,3]

The NOVA CRT 8 electrolyte analyzer provides, in addition to the actual measurements, values that are corrected for pH 7.4. The pH correction was not used in the present study because it has not been validated for dogs. Furthermore in-vivo pH changes, which may influence the ionized electrolyte concentration of a patient, are ignored by this correction. The actual measurements from an anaerobically-handled sample are considered to be the most accurate.[19]

In conclusion, the NOVA CRT 8 analyzer is suitable for clinic use. The concentration of $Ca_i$ in anaerobic, cooled serum samples remain stable long enough for samples to be sent to a reference laboratory. In contrast, the concentration of $Mg_i$ is relatively unstable even when cooled or frozen and should be measured within 8 hours of collection for accurate results. Our reference ranges determined for $Ca_i$ and $Mg_i$ can only be applied for measurements obtained with the NOVA CRT 8 analyzer.

[a] NOVA CRT 8 electrolyte analyzer, Nova Biomedical, Waltham, MA, USA.

[b] AVL 988/4 electrolyte analyzer, AVL, Medical Instruments, Graz, Austria.

[c] Microlyte 6, Kone Instruments, Finland.

[d] Vonderschmitt DJ, Evaluation CRT 8, 1994, laboratory for clinical chemistry, Universitaetsspital Zuerich, Switzerland, personal communication Vonderschmitt.

[e] NOVA 8 Reference Manual.

[f] Nova Chemistry Control, Nova Biomedical, Waltham, MA, USA.

[g] Calcium chloride 2-hydrate crystalline, Merk, Zurich, Switzerland.

[h] Magnesium chloride 6-hydrate crystalline, Merk, Zurich, Switzerland.

[i] Calcium Ion Standard Solution, 0.1 molar, $CaCl_2 \cdot 2H_2O$, Fluka Chemie, Buchs, Switzerland.

[j] Magnesium Ion Standard Solution, 0.1 molar, $MgCl_2 \cdot 6H_2O$, Fluka Chemie, Buchs, Switzerland.

[k] 5 ml tubes with push cap, polypropylen, Sarstedt AG, Sevelen, Switzerland.

[l] 3 cc syringe containing approx. 50 units of lyophilized lithium and zinc heparin, Ownes-BriGam Medical Company, Morganton, NC, USA.

[m] 1.5 ml Eppendorf microtubes with attached „safety cap“, polypropylene, Sarstedt AG, Sevelen, Switzerland.

[n] 0.5 ml micro tubes with screw cap, polypropylene, Sarstedt AG, Sevelen, Switzerland.

[o] 634 $Ca^{++}$/pH Analyzer, Ciba Corning Diagnostics GmbH, Fernwald, Germany.

REFERENCES

1. Bowers GN, Brassard C and Sena SF. Measurement of ionized calcium in serum with ion-selective electrodes: a mature technology that can meet the daily service needs. *Clin Chem* 1986; 32:1437-144.

2. Cao Z, Tongate C and Elin RJ. Evaluation of AVL988/4 analyzer for measurement of ionized magnesium and ionized calcium. *Scand J Clin Lab Invest* 2001;61:389-394.

3. Cecco SA, Hristova EN, Rehak NN, et al. Clinically important intermethod differences for physiologically abnormal ionized magnesium results. *Am J Clin Pathol* 1997;108 :564-569.

4. Chew DJ and Meuten DJ. Disorders of calcium and phosphorus metabolism. *Vet Clin North Am* 1982; 12:411-438.

5. Dhupa N, and Proulx J. Hypocalcemia and Hypomagnesemia. *Vet Clin North Am* 1998; 28:587-608.

6. Elin RJ, Hristova EN, Cecco SA, et al. Comparison of precision and effect of pH and calcium on the AVL and NOVA magnesium ion-selective electrodes. *Scand J Clin Lab Invest Suppl* 1996;224:203-210.

7. Hristova EN, Cecco S, Niemela JE, Rehak NN, et al. Analyzer-dependent differences in results for ionized calcium, ionized magnesium, sodium and pH. *Clin Chem* 1995; 41:1649-1653.

8. Huijgen HJ, Sanders R, Cecco SA, et al. Serum ionized magnesium: comparison of results obtained with three ion-selective analyzers. *Clin Chem Lab Med* 1999;37:465-470.

9. Kulpmann WR and Gerlach M. Relationship between ionized and total magnesium in serum. *Scand J Clin Lab Invest Suppl* 1996; 224:251-258.

10. Landt M, Hortin GL, Smith CH, et al. Interference in Ionized Calcium Measurements by Heparin Salts. *Clin Chem* 1994; 40:565-570.

11. Lyon ME, Guajardo M, Laha T, et al. Zinc heparin introduces a preanalytical error in the measurement of ionized calcium concentration. *Scand J Clin Lab Invest* 1995; 55:61-65.

12. Mann FA, Boon GD, Wagner-Mann CC, et al. Ionized and total magnesium concentrations in blood from dogs with naturally acquired parvoviral enteritis. *JAVMA* 1998; 212:1398-1401.

13. Markova V, Sirakova I, Tsvetkova T, et al. Evaluation of an ion-selective electrolyte analyser: Microlyte 6. *Folia Med (Plovdiv)* 1997; 39:34-38.

14. Mischke R, Hänies R, Lange K, et al. Influence of albumin concentration on the relation between the concentration of ionized calcium and total calcium in canine blood. *Dtsch tierärztl Wschr* 1996; 103:199-204.

15. Rasmussen H, Barrett P, Smallwood J, et al. Calcium ion as intracellular messenger and cellular toxin. *Environ Health Perspect* 1990; 84:17-25.

16. Rehak NN, Cecco SA, Niemela JE, et al. Linearity and stability of the AVL and Nova magnesium and calcium ion-selective electrodes. *Clin Chem* 1996; 42:880-887.

17. Ritter C, Ghahramani M, Marsoner HJ. More on the measurement of ionized magnesium in whole blood. *Scand J Clin Lab Invest Suppl* 1996; 197:275-280.

18. Rosol TJ, Chew DJ, Nagode LA, et al. Pathophysiology of calcium metabolism. *Vet Clin Pathol* 1995; 24:49-63.

19. Rosol TJ, Chew DJ, Nagode LA, et al. Disorders of Calcium. In: *DiBartola SP*, eds. Fluid Therapy in Small Animal Practice. 2nd ed. Philadelphia: WB Saunders Co, 2000; 108-162.

20. Schenk PA, Chew DJ, Brooks CL. Effects of storage on serum ionized calcium and pH values in clinically normal dogs. *Am J Vet Res* 1995; 56:304-307.

21. Szenci O, Brydl E and Bajcsy CA. Effect of storage on measurement of ionized calcium and acid-base variables in equine, bovine, ovine, and canine. venous blood. *JAVMA* 1991; 199:1167-1169.

22. Thode J. Actual ionized calcium and pH in blood collected in capillary or evacuated tubes. *Scand J Clin Lab Invest* 1986; 46:89-93

23. Thode J, Juul-Jorgensen B, Seibaek M, et al. Evaluation of an ionized magnesium-pH analyser – NOVA 8. *Scand J Clin Lab Invest* 1998; 58:127-133.

Table 1: Determination of precision in a measurement series and day-to-day precision

| Sample | | Within-run (n=10) | | Between-run (n=10*) | |
|---|---|---|---|---|---|
| | | CV [%] | (Mean ± SD [mmol/L]) | CV [%] | (Mean ± SD [mmol/L]) |
| $Ca_i$ | Level low[e] | 0.9 | (0.65 + 0.01) | 2.2 | (0.64 + 0.01) |
| | Level high[e] | 0.6 | (1.77 + 0.01) | 1.1 | (1.73 + 0.02) |
| | Serum | 0.9 | (1.12 + 0.01) | | |
| $Mg_i$ | Level low[e] | 0.8 | (0.39 + 0.003) | 2.3 | (0.38 + 0.01) |
| | Level high[e] | 0.5 | (1.55 + 0.007) | 1.9 | (1.42 + 0.03) |
| | Serum | 1.5 | (0.45 + 0.007) | | |

* performed on 10 consecutive days

[e] standard samples from the manufacturer of the NOVA CRT 8

CV coefficient of variation

SD standard deviation

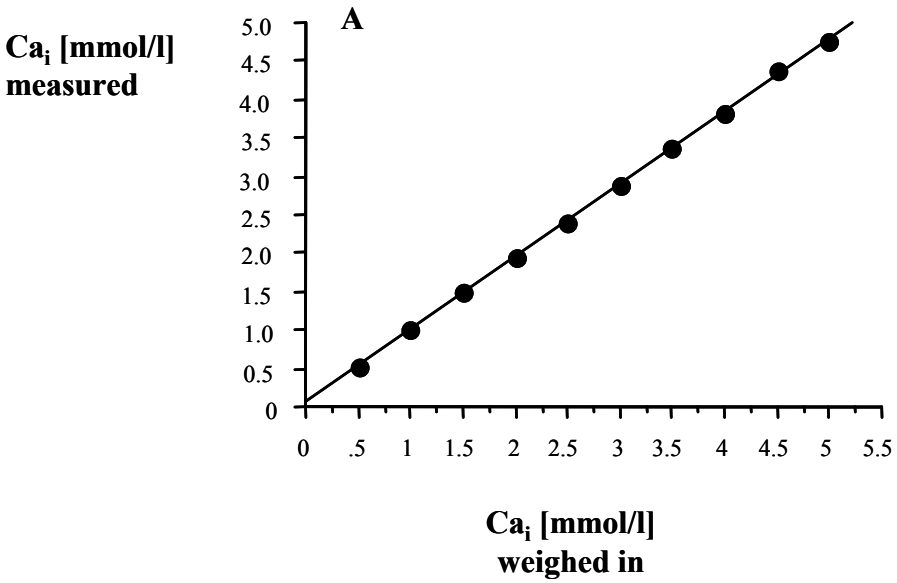
Table 2: The effect of different storage temperatures on the stability of ionized calcium and magnesium in anaerobically handled serum from 7 healthy dogs (values are means ± SD mmol/l).

| Time | Ionized Calcium at 22° C | Ionized Magnesium at 22° C |
|---|---|---|
| 0 | 1.314 ± 0.055 | 0.464 ± 0.063 |
| 1h | 1.311 ± 0.051 | 0.461 ± 0.058 |
| 2h | 1.313 ± 0.061 | 0.461 ± 0.060 |
| 4h | 1.327 ± 0.057 | 0.470 ± 0.061 |
| 8h | 1.301 ± 0.050 | 0.463 ± 0.061 |
| 24h | 1.300 ± 0.057 | **0.491 ± 0.067*** |
| 48h | **1.330 ± 0.066 *** | 0.474 ± 0.072 |
| | at 4° C | at 4° C |
| 0 | 1.333 ± 0.030 | 0.481 ± 0.030 |
| 24h | 1.351 ± 0.039 | **0.499 ± 0.030 *** |
| 48h | 1.341 ± 0.031 | 0.501 ± 0.032 * |
| 72h | **1.361 ± 0.032 *** | 0.529 ± 0.033 * |
| | at – 20° C | at – 20° C |
| 0 | 1.333 ± 0.030 | 0.481 ± 0.030 |
| 1w | 1.337 ± 0.049 | **0.526 ± 0.280 *** |
| 3w | 1.313 ± 0.047 | 0.491 ± 0.047 |
| 11w | 1.317 ± 0.067 | 0.513 ± 0.057 * |
| 26w | **1.173 ± 0.066 *** | 0.469 ± 0.034 |

* = significant difference, P≤0.05.

Bold values represent the first values that differed significantly from the values at times 0.

Figure 1: The linearity of measurements of different ionized calcium (A) and magnesium (B) aqueous solutions obtained with the NOVA CRT 8 analyzer. Direct measurements of the electrolyte concentrations were compared with actual weighed in amounts of $Ca_i$ or $Mg_i$ in aqueous solutions. The correlations and equations for the linear regressions for $Ca_i$ were y = 0.05 + 0.94 x, R = 1.00 and for $Mg_i$ were y = 0.03 + 0.88 x, R = 0.99.



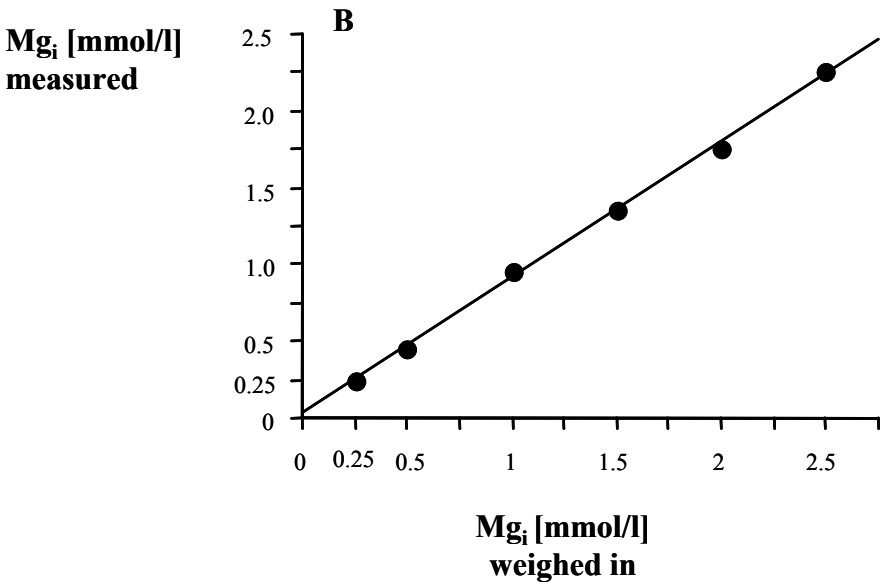
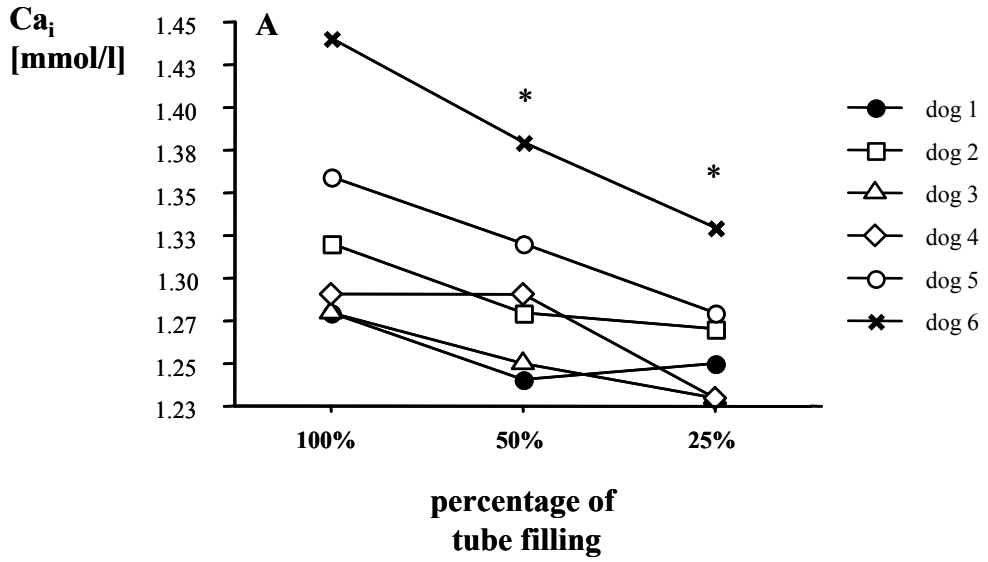



Figure 2: The effect of air on the ionized calcium (A) and magnesium (B). The serum from a blood sample from each of six dogs was divided into three tubes such that one tube was completely filled, one filled to 50% capacity and the third to 25% capacity. Twenty minutes later, the concentrations of ionized calcium and magnesium were determined and the results for each of the three tubes from one dog compared.

* The ionized electrolyte concentrations in tubes filled to 50% and 25% capacity were significantly lower than the concentration in tubes that were completely filled ($p \leq 0.05$; ANOVA)



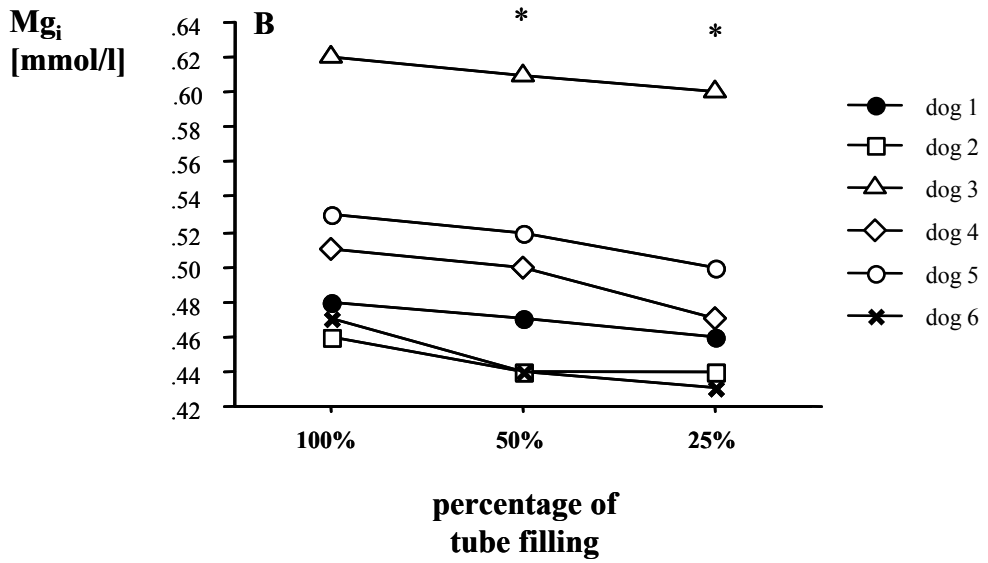
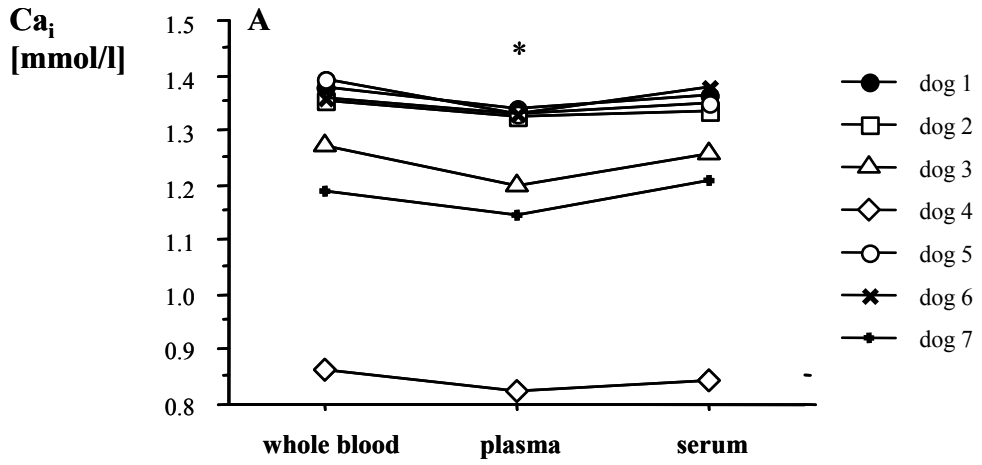



Figure 3: Comparison of the concentrations of ionized calcium (A) and magnesium (B) in whole blood, plasma and serum from 7 dogs.

* Measurement significantly lower ($p \leq 0.05$; ANOVA) than those of the other two sample types.



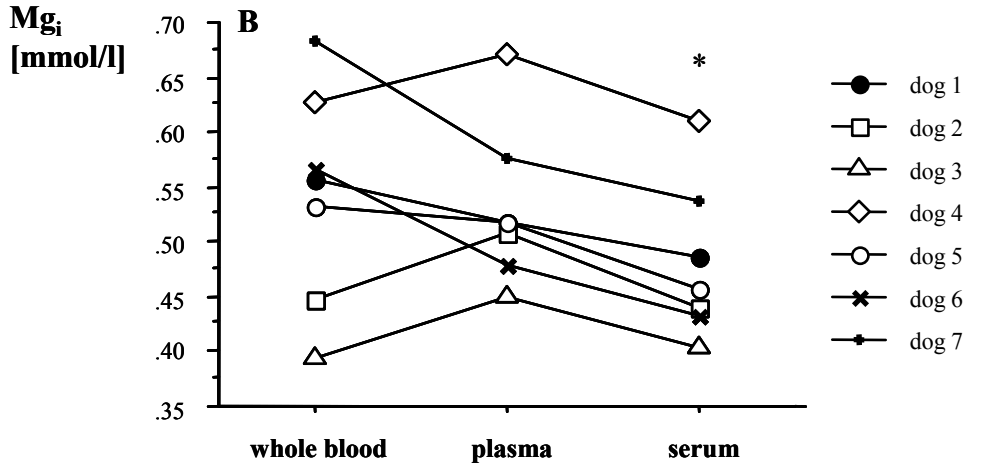
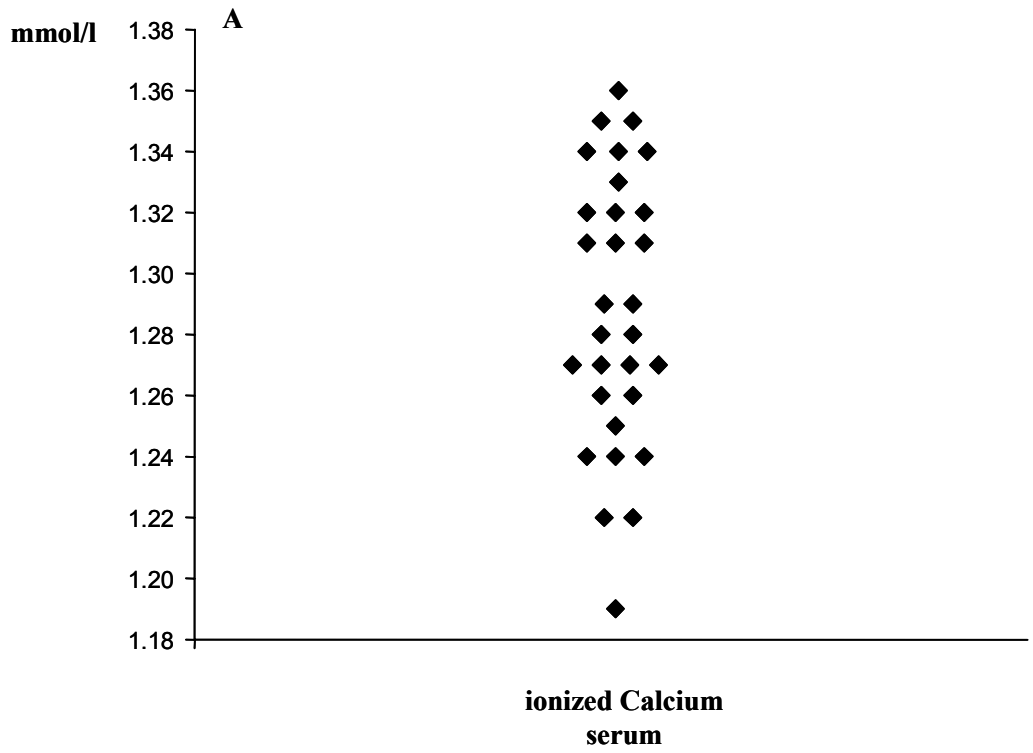
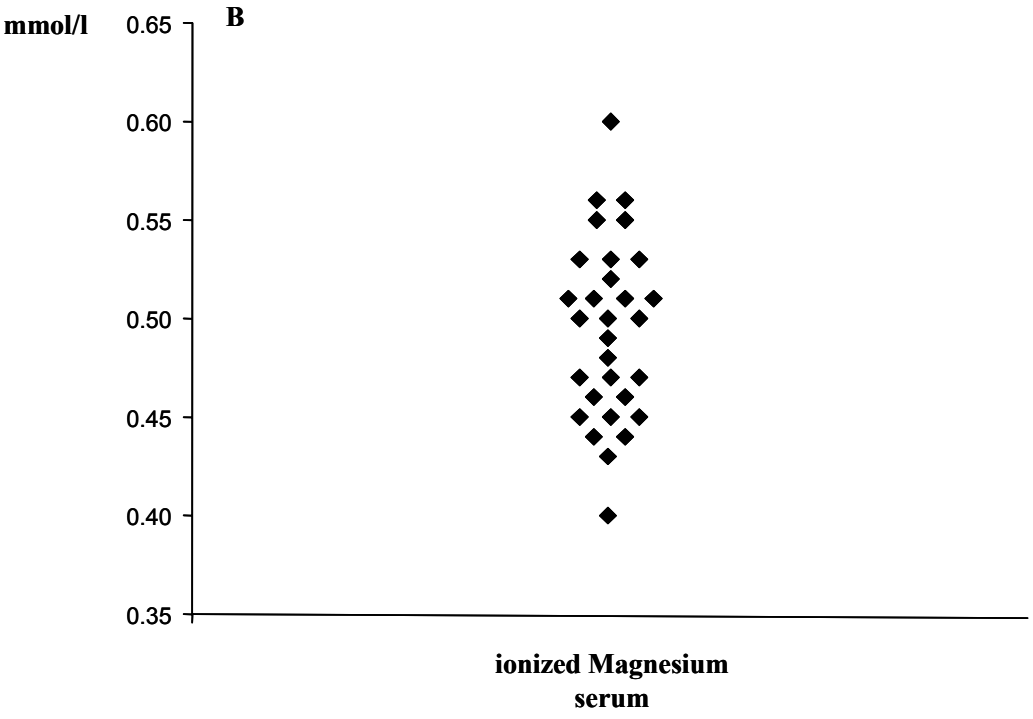



Figure 4: Scatter diagrams showing individual results of anaerobically handled serum ionized calcium (A) and magnesium (B) concentrations from 30 healthy dogs.



## PART 2:

## Evaluation of an electrolyte analyser for measurement of concentrations of ionised calcium and magnesium in cats

ABSTRACT

The goal of this study was to evaluate the NOVA CRT 8 electrolyte analyser for determination of concentrations of ionised calcium ($Ca_i$) and magnesium ($Mg_i$) in cats, to determine the effects of sample handling, to determine the stability of samples and to establish analyser-specific reference ranges for $Ca_i$ and $Mg_i$ in healthy cats. The precision, linearity and analytical accuracy of the NOVA CRT 8 analyser were good. The concentrations of $Ca_i$ and $Mg_i$ were significantly lower in aerobically handled serum samples than in those handled anaerobically. The concentrations of $Ca_i$ and $Mg_i$ differed significantly among whole blood, plasma and serum. In anaerobically handled serum, the concentration of $Ca_i$ was stable for 8 hours at 22ºC, for 5 days at 4ºC and for 1 week at -20ºC. In contrast, the concentration of $Mg_i$ was stable for 8 hours at 22ºC but for less than 24 hours at 4ºC and for less than 1 week at -20ºC. In anaerobically handled serum from 36 cats, the reference ranges were 1.20 – 1.35 mmol/L for $Ca_i$ and 0.47 – 0.59 mmol/L for $Mg_i$. The NOVA CRT 8 electrolyte analyser is suitable for determination of $Ca_i$ and $Mg_i$ concentrations in cats. Anaerobically handled serum samples stored at room temperature yield accurate results when analysed within 8 hours.

INTRODUCTION

Calcium and magnesium are two essential cations, which play an important role in numerous metabolic and cellular functions. Recent studies have investigated the role of these electrolytes in various feline disorders including idiopathic hypercalcaemia (Midkiff *et al*., 2000), calcium oxalate urolithiasis associated with hypercalcaemia (McClain *et* al., 1999; Savary, *et al*., 2000) and clinically relevant hypocalcaemia associated with pancreatitis (Kimmel *et al*., 2001), urethral obstruction (Drobatz and Hughes, 1997) and lymphoma involving large granular lymphocytes (Wellman *et al.*, 1992). The role of magnesium in intensive care medicine has received particular attention. In critically ill humans (Broner *et al.*, 1990; Rubeiz *et al.* 1993), dogs (Martin *et al.*, 1999) and cats (Toll *et al.*, 2002), abnormalities in magnesium concentration have been correlated with high rates of morbidity and mortality.

In blood, both electrolytes may be protein-bound, free or complexed with other substances. Only the free ionised fraction is biologically active and is maintained in a narrow concentration range by various interacting feedback loops (Rosol *et al*., 2000). For financial and technical reasons, most diagnostic laboratories measure only the total concentrations of calcium and magnesium. However, the serum concentrations of free calcium and magnesium may be affected by total serum protein concentration, serum pH, presence of carrier proteins and individual protein-binding affinity and thus, do not always correlate with the respective total concentrations (Chew *et al.*, 1989; Deniz and Mischke, 1995; Kulpmann and Gerlach, 1996; Rosol *et al.*, 2000; Jutkowitz *et al.*, 2002).

Therefore, it would be advantageous to directly measure the concentrations of ionised calcium ($Ca_i$) and magnesium ($Mg_i$) so that clinically relevant abnormalities are more reliably detected. Worldwide, there are only a few types of electrolyte analysers available for the measurement of the concentrations of $Ca_i$ and $Mg_i$, using ion-selective electrodes (Cao *et al.*,

2001). These analysers have been used in human medicine for a number of years (Bowers *et al.*,1986; Thode *et al.*, 1989; Zoppi *et al.*, 1996; Markova *et al.*, 1997; Hoshimo *et al.*, 2001) and to some extent in veterinary medicine (Deniz and Mischke, 1995; Drobatz and Hughes, 1997; Mann *et al.*, 1998; Kimmel *et al.*, 2001). To the authors' knowledge, this type of electrolyte analyser has not been evaluated for determination of $Ca_i$ and $Mg_i$ concentrations in cats.

The goals of this study were to evaluate the NOVA CRT 8 analyser for determination of $Ca_i$ and $Mg_i$ in healthy cats, to investigate various factors affecting measurements and to establish analyser-specific reference ranges for cats.

## MATERIALS AND METHODS

**Analyser -** The Nova CRT 8 electrolyte analyser is equipped with ion-selective electrodes and simultaneously measures haematocrit, pH and the concentrations of $Na^+$, $K^+$, $Mg^{++}$ and $Ca^{++}$ in whole blood, plasma and serum. The analyser provides calculated results for $Ca_i$ (measurement range 0.1 - 5.0 mmol/L) and $Mg_i$ (measurement range 0.1 – 2.5 mmol/L), normalised to pH 7.4. It also automatically corrects $Mg_i$ results for $Ca_i$ concentration. A sample volume of 180 µL is required, and the measurement cycle is 55 seconds.

**Cats -** Forty adult cats owned by employees of the Faculty of Veterinary Medicine, University of Zurich, were used for this study. The cats were considered healthy based on history and the results of physical examination, a complete blood count and biochemical profile. The cats belonged to four different breeds and ranged in age from half a year to 14 years (mean 5.5 years). There were 10 intact female, 8 spayed female, 10 intact male and 12

castrated male cats. Of the 40 cats, 20 were randomly selected to form three subgroups to study the effects of sample handling and storage conditions on electrolyte concentrations: samples from 6 cats were used to investigate the effect of air; samples from seven cats were used for comparison of different types of blood specimens and to determine serum sample stability at 22° C; and samples of another seven cats were used to study serum sample stability at 4° C and – 20°C. Of the 40 cats only those above one year of age, i.e. 36 cats, were used to establish reference ranges for $Ca_i$ and $Mg_i$ concentrations.

**Precision, linearity and analytical accuracy -** For the determination of precision in a measurement series, two standard samples from the manufacturer (Nova Chemistry Control, Nova Biomedical, Waltham, MA, USA) with a low ($Ca_i$, 0.59 to 0.83 mmol/L; $Mg_i$, 0.26 to 0.42 mmol/L) and a high concentration ($Ca_i$, 1.50 to 1.90 mmol/L; $Mg_i$, 1.32 to 1.68 mmol/L) of $Ca_i$ and $Mg_i$, and a feline serum sample with mid-range ($Ca_i$, 1.25 mmol/L; $Mg_i$, 0.43 mmol/L) concentrations of $Ca_i$ and $Mg_i$ were measured ten times within ten minutes. For day-to-day precision, the manufacturer's two standard samples with a low and a high concentration of $Ca_i$ and $Mg_i$ were measured every 24 hours for ten consecutive days. Samples were stored anaerobically at 4ºC.

For the determination of linearity, highly concentrated aqueous solutions of calcium (4.84 mmol/L) and magnesium (2.49 mmol/L) were prepared and diluted with isotonic saline solution to make 50%, 25%, 12.5% and 6.25% solutions. For each solution, the concentration of $Ca_i$ and $Mg_i$ was measured with the analyser and compared to the calculated values.
For determination of analytical accuracy, calcium and magnesium solutions of different concentrations ($Ca_i$, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 mmol/L; $Mg_i$, 0.25, 0.5, 1.0, 1.5, 2.0 and 2.5 mmol/L) were prepared and the concentrations measured. The

measurements obtained with the analyser were compared to the expected concentrations of the prepared solutions.

**Effect of air -** To determine how the exposure of specimens to air affects measurements, blood was collected from the jugular vein of six cats and immediately transferred into three polypropylene tubes (5-ml tubes with push cap, Sarstedt AG, Sevelen, CH) such that, respectively, 100%, 50% and 25% of the tubes was filled. In tubes that were completely filled (100%), inclusion of visible air bubbles was carefully avoided to achieve completely anaerobic conditions. Fifteen minutes later the samples were centrifuged at 7,500 x g for 2 - 5 minutes, followed immediately by measurement of concentrations of $Ca_i$ and $Mg_i$. The results of the tubes with the different air contents were compared.

**Comparison of $Ca_i$ and $Mg_i$ concentrations in whole blood, plasma and serum -** For determination of $Ca_i$ and $Mg_i$ concentrations in whole blood, samples were collected from seven cats into heparin-coated syringes (3-ml syringe containing approx. 50 units of lyophilised lithium and zinc heparin, Ownes-BriGam Medical Company, Morganton, NC, USA), and measurements were immediately made from the tip of the syringe. Thereafter, the remaining blood was placed in an Eppendorf serum tube (1.5-ml Eppendorf microtubes with attached "safety cap", polypropylene, Sarstedt AG, Sevelen, Switzerland) for harvesting plasma, avoiding the inclusion of visible air bubbles. Within 5 minutes, a second blood sample was collected from each cat into an Eppendorf serum tube without anticoagulant for harvesting serum. All tubes were allowed to sit for 15 minutes, after which they were centrifuged at 7,500 x g for 2 - 5 minutes, before $Ca_i$ and $Mg_i$ were measured in plasma and serum.

**Stability of samples stored anaerobically at different temperatures –** 0.5-ml aliquots of serum were placed in airtight tubes so that the tube was completely filled (0.5-ml micro tubes with screw cap, polypropylene, Sarstedt AG, Sevelen, Switzerland). Serum samples from 7 cats were stored at 22ºC. Serum samples from 7 different cats were stored at 4ºC and at -20ºC. For all three temperatures, the first measurement was performed immediately after centrifugation of the blood. For samples stored at 22ºC, additional measurements were made 1, 2, 3, 4, 8, 24 and 48 hours later. For samples stored at 4ºC, additional measurements were made every 24 hours for 5 days. For samples stored at -20ºC, additional measurements were made 1, 3, 11 and 26 weeks later. The frozen samples were thawed for 30 minutes at 22ºC before processing. The measurements obtained after storage were compared to the values of the first measurement. A sample was considered stable when the ANOVA for repeated measures did not reveal a significant difference from the first measurement.

**Reference values** - A blood sample was collected from the jugular vein of all 36 cats and placed into an Eppendorf serum tube (1.5-ml Eppendorf microtubes with attached "safety cap", polypropylene, Sarstedt AG, Sevelen, Switzerland). The tubes were filled completely, excluding any visible air bubbles, and closed tightly. After 10 to 15 minutes, the tubes were centrifuged at 7,500 x g for 2 - 5 minutes and the concentrations of $Ca_i$ and $Mg_i$ were immediately determined.

**Statistics -** Data were compiled using Excel 98 (Microsoft Inc.). Statistical analysis was performed using StatView 5.0 (SAS Inc., Wangen Dübendorf, Switzerland). Data were subjected to the QC-test for normality of the distribution. Precision was calculated using coefficients of variation. Linearity was assessed using regression analysis and correlation coefficients. ANOVA was used for multiple comparisons, and the Bonferroni-Dunn post test

was used for the comparison of individual means. Sample stability was analysed using ANOVA for repeated measures. The Bonferroni-Dunn post test was used for the comparison of individual values after storage to the value at the start of storage. Reference ranges were defined as the range from the 5th to the 95th percentile. Unless otherwise stated, values are reported as mean ± SD, and the unit is mmol/L. Differences were considered statistically significant at $P \leq 0.05$.

RESULTS

**Precision, linearity and analytical accuracy -** The coefficients of variation for precision in a measurement series for the different concentrations of $Ca_i$ and $Mg_i$ ranged from 0.5 to 0.8%. Those for day-to-day precision ranged from 1.1 to 2.3%.

For both $Ca_i$ and $Mg_i$, there was very good agreement between the calculated and measured concentrations for the entire measurement range of the analyser ($Ca_i$: R = 0.999; $Mg_i$: R = 0.998).

There was a linear relationship between the measured and expected concentrations of $Ca_i$ and $Mg_i$. The regression curves for $Ca_i$ and $Mg_i$ were, $y = 0.05 + 0.94x$ and $y = 0.03 + 0.88$ x, and the correlation coefficients were R = 1.00 and R = 0.99, respectively (Fig. 1).

**Effect of air** – The concentrations of $Ca_i$ in tubes filled to 25% capacity (1.26 ± 0.04 mmol/L) and 50% capacity (1.28 ± 0.04 mmol/L) and were significantly lower ($P<0.01$) than the concentration in tubes that were completely filled (1.32 ± 0.04 mmol/L). The same was true for $Mg_i$ with concentrations of 0.52 ± 0.06, 0.53 ± 0.06, and 0.55 ± 0.06 mmol/L, respectively ($P<0.01$, Fig 2).

**Comparison of $Ca_i$ and $Mg_i$ in whole blood, plasma and serum -** The concentration of $Ca_i$ was significantly lower in plasma (1.30 ± 0.04 mmol/L) than in whole blood (1.35 ±

0.04 mmol/L) and serum (1.33 ± 0.04 mmol/L). The concentration of $Mg_i$ was significantly lower in serum (0.60 ± 0.04 mmol/L) than in whole blood (0.66 ± 0.11 mmol/L) and plasma (0.67 ± 0.07 mmol/L); ($P < 0.01$; Fig 3).

**Stability of samples stored anaerobically at different temperatures** - The concentration of $Ca_i$ was stable for 8 hours at 22ºC, for 5 days at 4ºC and for 1 week at -20ºC. The concentration of $Mg_i$ was stable for 8 hours at 22ºC. After 24 hours at 4ºC and after 1 week at -20ºC, the concentration of $Mg_i$ was significantly higher than before storage (Table 1).

**Reference values** -The reference range using anaerobically handled serum was 1.20 – 1.35 mmol/L for $Ca_i$ and 0.47 – 0.59 mmol/L for $Mg_i$.

## DISCUSSION

This study demonstrated that the NOVA CRT 8 analyser is suitable for the measurement of $Ca_i$ and $Mg_i$ in cats. But our results, as well as those of other studies (Landt *et al.,* 1994; Schenk *et al.,*1995; Rosol *et al.,* 2000), have shown that special handling and storage of samples is critical for accurate results. In samples that are not handled anaerobically, carbon dioxide escapes, resulting in an increase in sample pH. Because there are fewer hydrogen ions available in these samples, the hydrogen binding sites on proteins become occupied by ionised electrolytes, resulting in decreased concentrations of $Ca_i$ and $Mg_i$ (Szenci *et al.*, 1991; Schenk *et al.,*1995; Rosol *et al.,* 2000). Our results demonstrated the importance of strict anaerobic handling of samples; serum samples stored in tubes that were only half full had significantly lower of $Ca_i$ and $Mg_i$ concentrations 20 minutes after preparation than had anaerobically handled samples.

Ionised calcium and magnesium can be measured in whole blood, plasma or serum. Harvesting whole blood and plasma requires an anticoagulant, which may affect the free electrolyte fraction in different ways. Anticoagulants may bind with ionised electrolytes and thereby reduce their concentration, or electrolytes of the anticoagulant may interfere with the electrode function or displace bound electrolytes from serum proteins and thus, result in increased values (Landt *et al.,* 1994; Swanson, 1994; Lyon *et al.,* 1995; Ritter *et al.,* 1996). In the present study, the concentration of $Ca_i$ was significantly lower in plasma than in serum, which was thought to be due to the formation of complexes of heparin and $Ca_i$ in plasma. Variations in pH were not likely to play a role in this difference because both serum and plasma samples underwent identical anaerobic handling. The plasma concentration of $Ca_I$ was also significantly lower than that of whole blood, although both specimens contained the same anticoagulant. This difference may have been attributable to a delay in the measurements in plasma. Measurements in whole blood were made immediately after collection and from the tip of the syringe, whereas those in plasma were performed a few minutes later. Possibly, this delay allowed for a larger portion of the heparin from the coated syringe to dissolve and to form complexes with the $Ca_i$.

In contrast, the concentration of $Mg_i$ was significantly lower in serum than in whole blood or plasma. Presumably, this was due to zinc ions in the anticoagulant used (Lyon, 1995; Ritter, 1996). Our results indicate that measurements differ among whole blood, plasma and serum and that the results of one cannot be substituted for another. We therefore recommend the use of serum for measurement of $Ca_i$ and $Mg_i$ because an anticoagulant is not required and serum samples remain stable for a longer period of time (Rosol *et al.*, 2000).

$Mg_i$ values obtained in serum samples of 6 cats used to compare influence of different types of blood, were slightly above the reference range. This may be explained by a significantly elevated $Mg_i$ level in two cats (0.65 and 0.68 mmol/l), which were used for this part of evaluation, but excluded from the reference range study because of their young age.

The results of this study indicate that for in-house analysis, serum does not need to be refrigerated because the concentrations of $Ca_i$ and $Mg_i$ remain stable at room temperature for 8 hours. Refrigeration (4ºC) is advised for serum samples if determination of $Ca_i$ concentration is not feasible within 8 hours. In contrast, the concentration of $Mg_i$ was significantly decreased after 24 hours of storage at 4ºC and after 1 week at -20ºC. Thus, reliable $Mg_i$ measurements in serum samples stored at room temperature can only be expected within 8 hours of collection. For a more accurate determination of the stability of $Mg_i$ in samples stored at 4ºC or -20ºC, measurements performed at shorter time intervals would be necessary.

Reference ranges should be determined according to the type of specimen (plasma, serum or whole blood), species and age of animal and type of analyser used. To our knowledge, reference ranges for serum $Ca_i$ and $Mg_i$ have not been performed in a large population of cats using the NOVA CRT 8. The results of a study involving a small number of cats and the NOVA CRT analyser (Wooldridge and Gregory, 1999: 10 cats, reference ranges, $Ca_i$ = 1.19 – 1.27 mmol/L, $Mg_i$ = 0.45 – 0.53 mmol/L) were in good agreement with those of the present study. However, reference ranges determined in cats (Kallfelz *et al.*, 1980; Lewis *et al.,* 1978) and humans (Hristova *et al.,* 1995; Rehak *et al.,* 1996; Cecco *et al.,* 1997; Huijgen *et al.,* 1999) using different electrolyte analysers have shown that there are marked analyser-specific differences. If correction for these differences is not feasible, analyser-specific reference ranges should be used.

In addition to the actual measurements, the NOVA CRT 8 electrolyte analyser provides values that are corrected for pH 7.4. The pH correction was not used in the present study because it has not been validated for cats. Changes in pH occurring *in vivo*, which may influence the ionised electrolyte concentration of a patient, are ignored by this correction. The actual measurements from an anaerobically-handled sample are considered to be the most accurate (Rosol *et al.*, 2000)

In conclusion, the NOVA CRT 8 analyser is suitable for clinic use. The concentration of $Ca_i$ in anaerobically-handled, cooled serum samples remains stable long enough for shipment to a laboratory. In contrast, the concentration of $Mg_i$ is less stable, even at lower temperatures, and should be measured within 8 hours of collection for accurate results. The reference ranges determined here for $Ca_i$ and $Mg_i$ apply only to the NOVA CRT 8 analyser.

REFERENCES

Bowers, G.N., Brassard, C. and Sena, S.F., 1986. Measurement of ionized calcium in serum with ion-selective electrodes: a mature technology that can meet the daily service needs, Clinical Chemistry, 32, 1437-144

Broner, C.W., Stidham, G.L., Westkirchner, D.F. and Tolley E.A., 1990. Hypermagnesemia and hypocalcemia as predictors of high mortality in critically ill pediatric patients, Critical Care Medicine, 18, 921-928

Cecco, S.A., Hristova, E.N., Rehak, N.N. and Elin, R.J., 1997. Clinically important intermethod differences for physiologically abnormal ionized magnesium results, American journal of clinical pathology, 108, 564-569

Chew, D.J., Leonard, M. and Muir, W., 1989. Effect of sodium bicarbonate infusions on ionized and total calcium concentrations in serum of clinically normal cats, American Journal of Veterinary Research, 50, 145-150

Deniz, A., Mischke R., 1995. Ionisiertes Calcium und Gesamtcalcium bei der Katze, Berliner und Münchner Tierärztliche Wochenschrift, 108, 105-108

Drobatz, K.J. and Hughes, D., 1997. Concentration of ionized calcium in plasma from cats with urthral obstruction, Journal of American Veterinary Medical Association, 211, 1392-1395

Hristova, E.N., Cecco, S., Niemela, J.E., Rehak, N.N. and Elin, R.J., 1995. Analyzer-dependent differences in results for ionized calcium, ionized magnesium, sodium and pH, Clinical Chemistry, 41, 1649-1653

Huijgen, H.J., Sanders, R., Cecco, S.A., Rehak, N.N., Sanders, G.T. and Elin, R.J., 1999. Serum ionized magnesium: comparison of results obtained with three ion-selective analysers, Clinical Chemistry and Laboratory Medicine, 37, 465-470

Jutkowitz, L.A., Rozanski, E.A., Moreau, J.A. and Rush, J.E., 2002. Massive transfusion in dogs: 15 cases (1997-2001), Journal of American Veterinary Medical Association, 220,1664-1669

Kallfelz, F.A., Bressett, J.D. and Wallace, R.J., 1980. Urethral obstruction in random source and SPF male cats induced by high levels of dietary magnesium or magnesium and phosphorus, Feline Practice, 10, 25-35

Kimmel, S.E., Washabau, R.J. and Drobatz, K.J., 2001. Incidence and prognostic value of low plasma ionized calcium concentration in cats with acute pancreatitis: 46 cases (1996-1998), Journal of American Veterinary Medical Association, 219, 1105-1109

Landt, M., Hortin, G.L., Smith. C.H., McClellan, A., and Scott, M.G., 1994. Interference in Ionized Calcium Measurements by Heparin Salts, Clinical Chemistry, 40, 565-570

Lewis, L.D., Chow, F.H.C., Tanton, G.F. and Hamar D.W., 1978. Effekt of various dietary mineral concentrations on the occurrence of feline urolithiasis, Journal of American Veterinary Medical Association, 172, 559-563

Lyon, M.E., Guajardo, M., Laha, T., Malik, S., Henderson, P.J. and Kenny, M.A., 1995. Zinc heparin introduces a preanalytical error in the measurement of ionized calcium concentration, Scandinavian Journal of Clinical and Laboraory Investigation, 55, 61-65

Mann, F.A., Boon, G.D., Wagner-Mann, C.C., Ruben, D.S., and Harrington, D.P., 1998. Ionized and total magnesium concentrations in blood from dogs with naturally acquired parvoviral enteritis, Journal of American Veterinary Medical Association, 212:1398-1401

Martin, L. G., Matteson, V.L., Wingfield, W.E., Van Pelt, D.R. and Hackett T.B., 1999. Abnormalities of Serum Magnesium in Critically Ill Dogs: Incidence and Implications, Journal of Veterinary Emergency and Critical Care, 4, 15-20

McClain, H.M., Barsanti, J.A. and Bartges, J.W., 1999. Hypercalcemia and calcium oxalate urolithiasis in cats: a report of five cases, Journal of American Animal Hospital Association, 35, 297-301

Midkiff, A.M., Chew, D.J., Randolph, J.F., Center, S.A. and DiBartola S.P., 2000. Idiopathic Hypercalcemia in Cats, Journal of Veterinary Internal Medicine, 14, 619-626

Rehak, N.N., Cecco, S.A., Niemela J.E., Hristova, E.N. and Elin, R.J., 1996. Linearity and stability of the AVL and Nova magnesium and calcium ion-selective electrodes, Clinical Chemistry, 42, 880-887

Ritter, C., Ghahramani, M. and Marsoner, H.J., 1996. More on the measurement of ionized magnesium in whole blood**,** Scandinavian Journal of Clinical and Laboraory Investigation. Supplementum**,** 197, 275-280

Rosol, T.J., Chew, D.J., Nagode, L.A. and Schenk, P., 2000. Disorders of Calcium. In: Fluid Therapy in Small Animal Practice, 2nd edn, (WB Saunders, Philadelphia), 108-162

Rubeiz, G.J., Thill-Baharozian, M., Hardie, D. and Carlson, R.W., 1993. Association of hypomagnesemia and mortality in acutely ill medical patients, Critical Care Medicine, 22, 372-373

Savary, K.C.M., Price, G.S. and Vaden, S.L., 2000. Hypercalcemia in Cats: A Retrospective Study of 71 Cases (1991-1997), Journal of Veterinary Internal Medicine, 14, 184-189

Schenk, P.A., Chew, D.J., Brooks, C.L., 1995. Effects of storage on serum ionized calcium and pH values in clinically normal dogs, American Journal of Veterinary Research, 56,304-307

Swanson, J.R., Heeter, C., Limbocker, M. and Sullivan M., 1994. Bias of Ionized Calcium Results from Blood Gas Syringes, Clinical Chemistry, 40, 669-670

Szenci, O., Brydl, E. and Bajcsy, C.A., 1991. Effect of storage on measurement of ionized calcium and acid-base variables in equine, bovine, ovine, and canine venous blood, Journal of American Veterinary Medical Association, 199, 1167-1169

Toll, J., Erb, H., Birnbaum, N. and Schermerhorn. T., 2002. Prevalence and Incidence of Serum Magnesium Abnormalities in Hospitalized Cats, Journal of Veterinary Internal Medicine, 16, 217-221

Wellman, M.L., Hammer, A.S., Di Bartola, S.P., Carothers, M.A., Kociba, G.J. and Rojko, J.L., 1992. Lymphoma involving large granular lymphocytes in cats: 11 cases (1982-1991), Journal of American Veterinary Medical Association, 201, 1265-1269

Wooldridge, J.D. and Gregory, C.R., 1999. Ionized and Total Serum Magnesium Concentrations in Feline Renal Transplant Recipients, Veterinary Surgery, 28, 31-37

Zoppi, F., De Gasperi, A., Guagnellini, E., Marocchi, A., Mineo, E., Pazzucconi, F., Rossi, C. and Turrini, D., 1996. Measurement of ionized magnesium with AVL 988/4 electrolyte analyzer: preliminary analytical and clinical results, Scandinavian Journal of Clinical and Laboraory Investigation, 224: 259-274

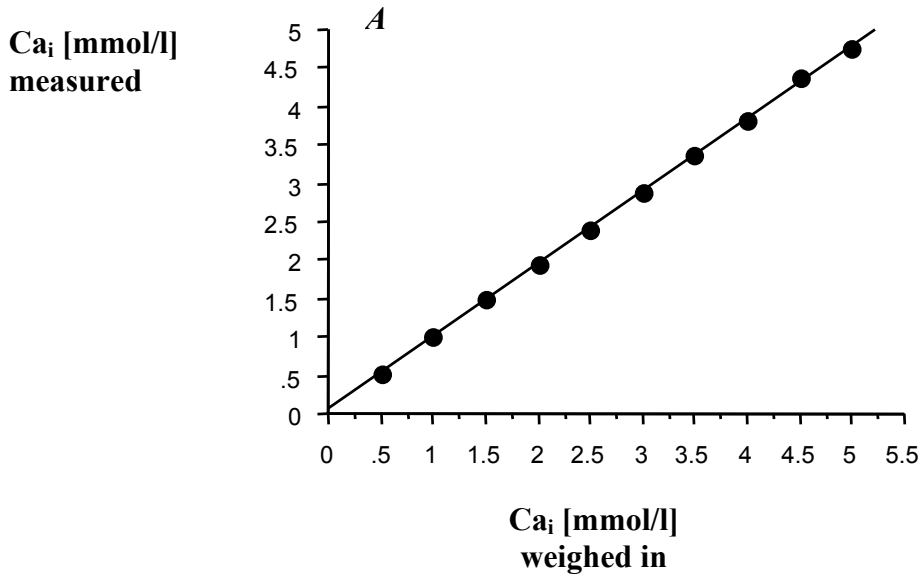
Table 1: The effect of different storage temperatures on the stability of ionised calcium and magnesium in anaerobically handled serum from 7 healthy cats (values are means ± SD mmol/l).

| Time | Ionised Calcium at 22° C | Ionised Magnesium at 22° C |
|---|---|---|
| 0 | 1.271 ± 0.058 | 0.437 ± 0.082 |
| 1h | 1.270 ± 0.055 | 0.434 ± 0.088 |
| 2h | 1.274 ± 0.049 | 0.437 ± 0.085 |
| 4h | 1.271 ± 0.048 | 0.440 ± 0.087 |
| 8h | 1.273 ± 0.063 | 0.447 ± 0.087 |
| 24h | **1.251 ± 0.051*** | **0.476 ± 0.091*** |
| 48h | -------- | -------- |
| | at 4° C | at 4° C |
| 0 | 1.299 ± 0.047 | 0.540 ± 0.052 |
| 24h | 1.313 ± 0.043 | **0.563 ± 0.054 *** |
| 48h | 1.301 ± 0.043 | -------- |
| 72h | 1.299 ± 0.039 | -------- |
| 96h | 1.294 ± 0.042 | -------- |
| 120h | 1.311 ± 0.053 | -------- |
| | at – 20° C | at – 20° C |
| 0 | 1.299 ± 0.047 | 0.540 ± 0.052 |
| 1w | 1.306 ± 0.052 | **0.601 ± 0.068 *** |
| 3w | **1.233 ± 0.052*** | -------- |
| 11w | -------- | -------- |
| 26w | -------- | -------- |

***** = significant difference, P≤0.05.

Bold values represent the first values that differed significantly from the values at times 0.

Figure 1: The linearity of measurements of different ionised calcium (*A*) and magnesium (*B*) aqueous solutions obtained with the NOVA CRT 8 analyser. Direct measurements of the elecrolyte concentrations were compared with actual weighed in amounts of $Ca_i$ or $Mg_i$ in aqueous solutions. The correlations and equations for the linear regressions for $Ca_i$ were y = 0.05 + 0.94 x, R = 1.00 and for $Mg_i$ were y = 0.03 + 0.88 x, R = 0.99.



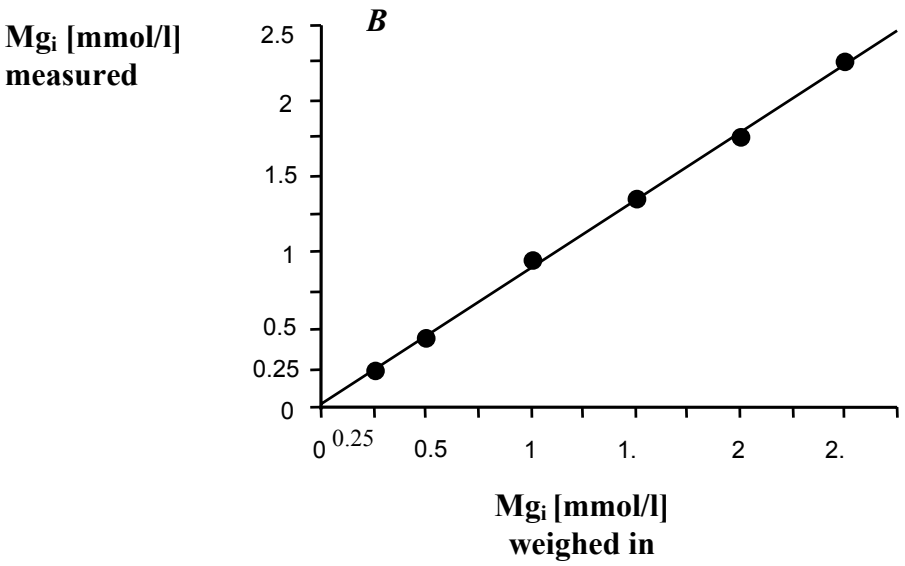



Figure 2: The effect of air on the ionised calcium (*A*) and magnesium (*B*). The serum from a blood sample from each of six cats was divided into three tubes such that one tube was completely filled, one filled to 50% capacity and the third to 25% capacity. Twenty minutes later, the concentrations of ionised calcium and magnesium were determined and the results for each of the three tubes from one cat compared. An asterix represents measurements that differ significantly ($p \leq 0.05$; ANOVA) from that of the tube that was completely filled with serum.

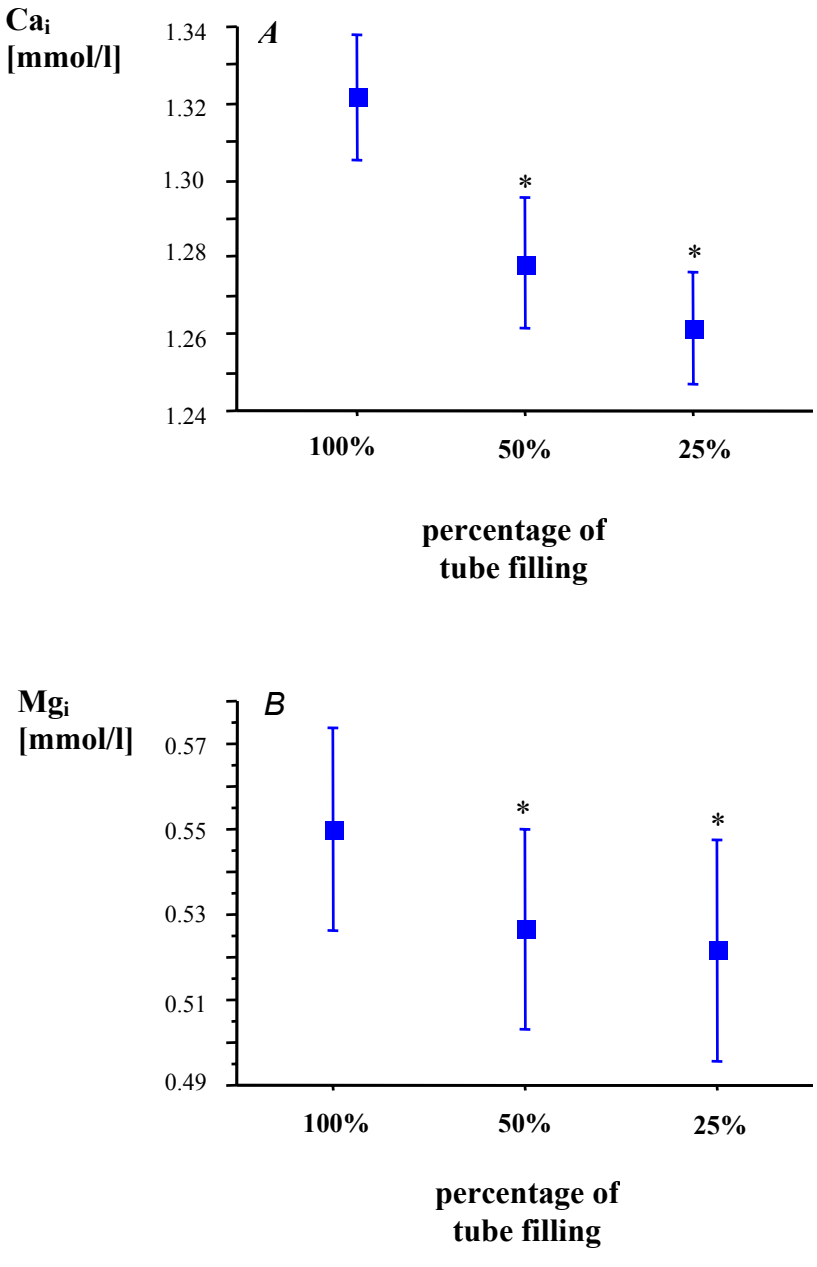
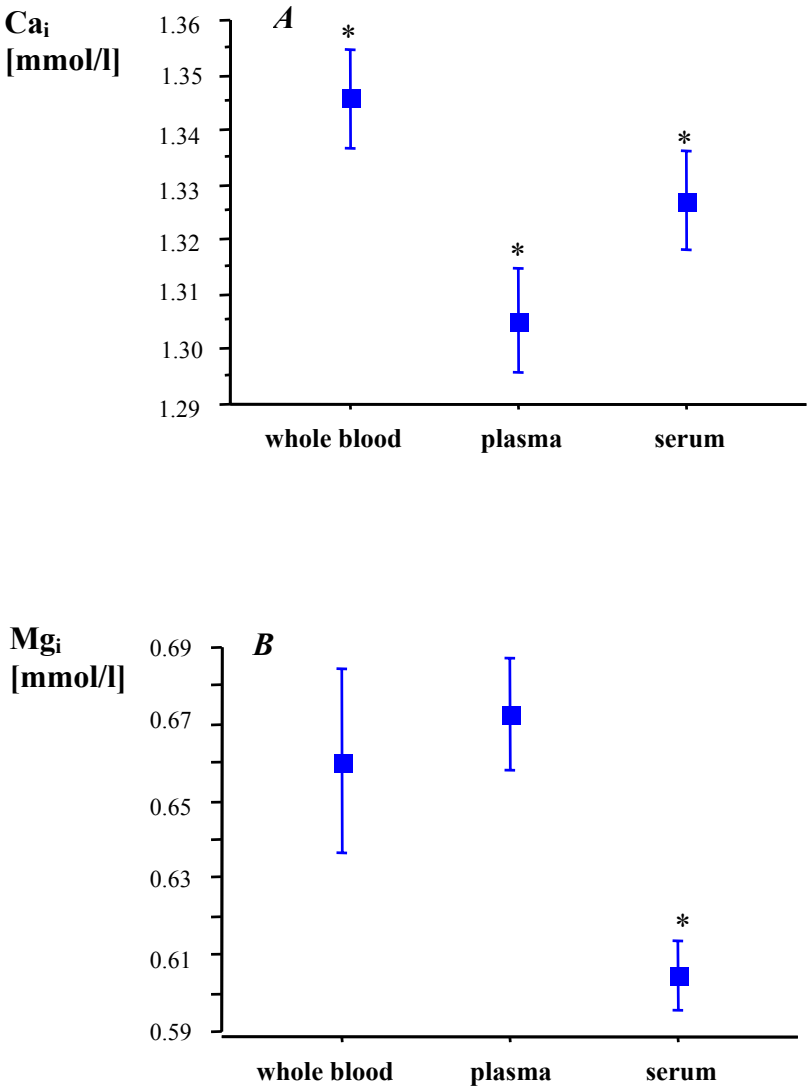


Figure 3: Comparison of the concentrations of ionised calcium (*A*) and magnesium (*B*) in whole blood, plasma and serum from 7 cats. An asterix represents measurements that were significantly lower ($p \leq 0.05$; ANOVA) than those of the other two sample types.



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## Lebenslauf

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